

Are *Tricholomopsis* spp. common or rare? – new data from Greece

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ABSTRACT—This study reports the presence of *Tricholomopsis flammula* and *T. sulfureoides* in Greece, providing additional data on the morphology, phylogeny, distribution, and rarity of these species.

KEY WORDS—*Agaricales*, macromycetes, wood-inhabiting fungi, molecular analysis

Introduction

Tricholomopsis Singer comprises saprotrophic and mostly wood-inhabiting species with tricholomatoid fruitbodies that typically possess yellow color overall and fibrils or squamules on pileus surface that may be differently colored. The basidiospores are smooth, non-amyloid and white in deposit, and the large cheilocystidia form a sterile lamellae edge. It is distributed mainly in the Northern Hemisphere (Smith 1960; Holec 2012a; Hosen & al. 2020). The genus includes c. 50 names worldwide (<http://www.indexfungorum.org>). In Europe, *Tricholomopsis* is represented by a small number of species, while in literature, until quite recently, there were contradictory opinions about the number and identity of the species. Studies that combine both traditional morphological and molecular methods have confirmed the presence of six species in Europe, *T. rutilans* (Schaeff.) Singer, *T. decora* (Fr.) Singer, *T. flammula*, *T. sulfureoides*, *T. pteridiicola* Olariaga & al., and *T. badinensis* Holec & al. (Holec & Kolařík 2011, 2012; Olariaga & al. 2015; Holec & al. 2019). *Tricholomopsis rutilans* and

T. decora are characterized as common species in Europe, whereas *T. flammula*, *T. sulfureoides*, *T. pteridiicola*, and *T. badinensis* are considered rare with few recordings. The first finding of *T. sulfureoides* in Europe came from Estonia and was initially described as a new species, *T. osiliensis* (Vauras 2009). Three years later, Holec (2012b) reported the presence of the same species in Slovakia. Further studies showed that *T. osiliensis* was conspecific with *T. sulfureoides*, a species already known from N. America (Vauras & al. 2012). Saar & Voitek (2015) confirmed this synonymy by studying the type collection of *T. sulfureoides*. Holec & al. (2019) documented the existence of *T. sulfureoides* in Poland as well. *Tricholomopsis flammula*, which is also considered a rare species, has a wide distribution in Europe and it is also found in Pakistan (Holec 2009; Holec & Kolařík 2011, 2012; Razaq & al. 2012). *Tricholomopsis pteridiicola* is a recently described new species collected from Spain and France and associated with the fern *Pteridium aquilinum*, in contrast with all other European members of the genus which grow, like most species of *Tricholomopsis*, on conifer wood (Olariaga & al. 2015). *Tricholomopsis badinensis* is a newly described species, known until now only from Slovakia (Holec & al. 2019). *Tricholomopsis badinensis* along with *T. decora* and *T. sulfureoides* have in common the absence of red or violet coloration on the basidiomata.

Recent collections as well as old represented by dried specimens preserved unidentified in the Mycetothea ATHUM were determined as the rare species *T. flammula* and *T. sulfureoides*. The aim of this study is to communicate the presence of *T. flammula* and *T. sulfureoides* in Greece, to confirm the variability of the species, and to add data on their morphology, phylogeny, distribution, and rarity.

Materials and methods

Morphological study

The studied material includes six collections of *T. flammula* and three of *T. sulfureoides* from different regions of Greece. Some of them were newly collected during the period 2012–15 from three National Parks of Greece (Mt. Ainos, Mt. Oiti, and Mt. Parnassos), while the rest were dried specimens preserved at the Dried Specimen Collection of the Mycetothea ATHUM, National & Kapodistrian University of Athens. For comparison purposes, six collections of *T. rutilans* were studied additionally.

Macromorphological descriptions of *T. flammula* and *T. sulfureoides* are based on detailed observation of the newly collected specimens, as well as on descriptions accompanying the dried specimens. Colors were described in daylight conditions and color codes refer to Kornerup & Wanscher (1981).

Microscopic observations were made from dried material using a Zeiss AxioImager A1 Differential Interference Contrast (DIC) microscope. Sections were mounted in Melzer's reagent for observation, measurements and photography. Spores were measured in side-view (25 spores per specimen). Spore sizes are presented as a central range, calculated as: mean $\pm 2 \times$ SD, flanked by parenthetical extreme values lying outside the range, and followed by mean length (L_m) and width (W_m). The length/width quotient (Q) is presented as the mean of all quotients, flanked by the minimum and maximum individual quotients.

Terminology used for description of macromorphological and micromorphological characteristics is in accordance with Vellinga (1988).

All specimens are deposited at the Dried Specimen Collection of the Mycetotheca ATHUM, National & Kapodistrian University of Athens, Greece.

Molecular analysis

Total genomic DNA was extracted from basidiocarps following the CTAB protocol of Winnepenninckx & al. (1993) as modified by Parmakelis & al (2003). Pieces from the flesh and lamellae of the pilei were placed in 700 μ l 2 $\times$ CTAB containing 20 μ l proteinase-K (10 mg/ml). The mixture was incubated overnight in a thermoshaker at 840 rpm and 56°C. Proteins were removed with chloroform alone. DNA was precipitated with ice-cold absolute ethanol, washed with 70% ice-cold ethanol and suspended with 50 μ l nanopure water.

The Internal Transcribed Spacer 1 and 2 with the intervening 5.8S nrDNA (ITS region) was amplified with the oligonucleotide primer sets 18S-ITS1/28S-ITS2 (Pantou & al. 2005). PCR was performed in a 25 μ l volume, in which 1 μ l of template DNA was mixed with 0.2 mM dNTPs, 0.4 mM of each primer and 0.5 units of Taq polymerase. The concentration of the $MgCl_2$ was 3.5 mM. The target loci were amplified with a cycle program comprising an initial denaturation step at 95°C for 5min, followed by 35 cycles of 30 sec at 94°C, 45 sec at 48°C, and 2 min at 72°C, and ending with 7 min sequence extension at 72°C. Automated sequencing of both strands of the PCR amplicons was performed in a PE-ABI3740 automated sequencer (using Big-Dye terminator chemistry). The primers in the sequencing reactions were the same as in the PCR amplifications. All fragments were read in both directions. Consensus sequences were computed from forward and reverse sequences using Codon-Code Aligner (v. 4.1.1, Codon Code Corporation) and were deposited in the GenBank database under accession numbers from MT981245 to MT981251 (TABLE 1).

The authenticity of the DNA sequences and the homology with the target genetic locus were evaluated by a BLAST search in the NCBI genetic database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The generated *Tricholomopsis* sequences, along with reference sequences obtained from NCBI and UNITE databases (TABLE 1) were assembled and aligned using Codon-Code Aligner (v. 4.1.1, Codon Code Corporation).

Phylogenetic trees based on ITS were constructed using Neighbor Joining (NJ) with MEGA 10.0 (Kumar & al. 2016), Maximum Likelihood (ML) using RAxML-HPC2 on XSEDE 8.2.10 (Stamatakis 2014) through the CIPRES Science Gateway V.3.3 (Miller & al. 2010) and Bayesian Inference (BI) with MrBayes v. 3.2.7 (Ronquist & al. 2012).

TABLE 1. Specimens and sequences used in the phylogenetic analysis. New sequences are given in bold. [HT]= holotype; [ST] = syntype.

SPECIES	VOUCHER	GENBANK (ITS)	COUNTRY	REFERENCE
<i>T. aurea</i>	SFSU: DED 8327	MF100960	Africa	Desjardin & Perry 2017
	SFSU: BAP 618	MF100961	Africa	Desjardin & Perry 2017
<i>T. badinensis</i>	PRM 946195	LS992163	Slovakia	Holec & al. 2019
	PRM 899423	LS992164	Slovakia	Holec & al. 2019
	PRM 946194	LS992165	Slovakia	Holec & al. 2019
<i>T. decora</i>	UBC F23918	KJ146733	Canada	Berbee & al. (unpublished)
	TUF 106634	UDB011838	Estonia	Saar & Voitk 2015
	UBC F23917	KJ146732	Canada	Berbee & al. (unpublished)
	PRM 899160	HE649942	Slovakia	Holec & Kolařík 2012
	TENN 021494	KP453708	USA	Sanchez-Garcia (unpublished)
<i>T. decora</i> [as <i>T. sulphureoides</i>]	NYSf 3116.A[HT]	UDB018405	USA	Saar & Voitk 2015
<i>T. flammula</i>	S-F-156625	KP058975	Sweden	Olariaga & al. 2015
	SR 161	FR822742	Pakistan	Razaq & al. 2012
	ARAN-Fungi 00322	KP058973	Spain	Olariaga & al. 2015
	WU 25091	FN554892	Austria	Holec 2009
	PRM 909608	FN554896	Czech Repub.	Holec & Kolařík 2011
	WU 12087	FN554897	Austria	Holec & Kolařík 2011
	TUF 118261	UDB015434	Estonia	Saar & Voitk 2015
	PRM 899459	FN554894	Czech Repub.	Holec & Kolařík 2011
	PRM 899162	HE649939	Slovakia	Holec & Kolařík 2012
	PRM 899190	HE649941	Slovakia	Holec & Kolařík 2012
	PRM 899180	HE649940	Slovakia	Holec & Kolařík 2011
	WU 13075	HE652866	Austria	Holec & Kolařík 2012
	PRM 899108	FN554893	Czech Repub.	Holec 2009, Holec & Kolařík 2011
	ATHUM 9529	MT981245	Greece	This study
	ATHUM 9528	MT981246	Greece	This study
<i>T. flavescens</i>	NYSf 1195.1 [ST]	UDB022699	USA	Saar & Voitk 2015

SPECIES	VOUCHER	GENBANK (ITS)	COUNTRY	REFERENCE
<i>T. ornaticeps</i>	PDD 82501	KY010820	New Zealand	Cooper & Park 2016
	PDD 102517	KY010822	New Zealand	Cooper & Park 2016
	PDD 102769	KY010824	New Zealand	Cooper & Park 2016
<i>T. pteridiicola</i>	ARAN-Fungi 00121	KP058988	Spain	Olariaga & al. 2015
	ARAN-Fungi 00321	KP058992	France	Olariaga & al. 2015
	ARAN-Fungi 00122	KP058990	Spain	Olariaga & al. 2015
<i>T. rutilans</i>	PRM 889120	FN554895	Slovakia	Holec 2009
	BIO-Fungi 11313	KP058979	Sweden	Olariaga & al. 2015
	H 6012126	UDB031626	Finland	FinBOL project (unpublished)
	PRM 899460	HE649946	Czech Republic	Holec & Kolařík 2012
	TUF 106244	UDB011443	Estonia	Saar & Voitk 2015
	UBC F16251	EF530929	Canada	Denis & al. (unpublished)
	ATHUM 7713	MT981249	Greece	This study
	ATHUM 7714	MT981250	Greece	This study
	ATHUM 9524	MT981251	Greece	This study
<i>T. rutilans</i> var. <i>splendidissima</i>	LIP 86127	KP058995	France	Olariaga & al. 2015
<i>T. aff. rutilans</i>	ARAN-Fungi 00323	KP058982	Spain	Olariaga & al. 2015
	UPS F- 646220	KP058984	Sweden	Olariaga & al. 2015
	TUB 011582	KP058981	Germany	Olariaga & al. 2015
<i>T. scabra</i>	PDD 102100	KY010821	New Zealand	Cooper & Park 2016
	PDD 102579 [HT]	KY010823	New Zealand	Cooper & Park 2016
<i>T. sulfureoides</i>	NYSf 3116.10 [HT]	UDB023125	USA	Saar & Voitk 2015
	PRM 945185	LT984728	Poland	Kolařík (direct submission)
	ATHUM 9527	MT981247	Greece	This study
	ATHUM 9526	MT981248	Greece	This study
<i>T. sulfureoides</i> [as <i>T. osiliensis</i>]	TUF 101571	UDB015070	Estonia	Saar & Voitk 2015
	TUF 101571	HE649944	Estonia	Holec & Kolařík 2012
<i>Pluteus romellii</i>	LE 217944	FJ774073	Russia	Malysheva & al. 2009

NJ analysis was conducted with a heuristic search with the following settings. The substitution model was Kimura 2 parameter with substitutions including transitions and transversions. Bootstrap support values were derived from 1000 replications and are indicated at the branching nodes. For ML the General Time Reversible (GTR) substitution model with gamma distribution and invariable sites was used and bootstrap supports were estimated by 1000 replicates. A Markov Chain Monte Carlo (MCMC) algorithm was used to generate the phylogenetic tree for the ITS genetic locus. The GTR substitution model with gamma distribution and invariable sites was chosen after testing in MEGA. The analysis was run in duplicate with four MCMC chains for 10,000,000 generations and stopped when the average standard deviation of split frequencies reached below 0.01. Random trees were sampled every 100 generations. Burnin was set to 25% after which the likelihood values were stationary. The species *Pluteus romellii* FJ774073 of the related family *Pluteaceae* was used to root the tree. The resulting trees were viewed with TreeView v. 1.6.6 and, together with the alignments, deposited into TreeBASE (<http://www.treebase.org>) under submission ID 27845.

Results

Morphological study

Tricholomopsis flammula Métrod ex Holec, J. Nat. Mus. (Prague), Nat. Hist. Ser.

178: 8 (2009)

FIG. 1

PILEUS 30–60 mm, first hemispherical to convex, at maturity plano-convex with a broad umbo, center sometimes depressed, surface pale yellow (2A3, 3A3), light yellow (2A5) to pastel yellow (3A4), covered with tiny greyish magenta (13E5, 14E5), greyish violet (18E6–7, 18D6, 16E6–7, 16D5, 15E6–7, 15D5–6) to dark purple (14F5–6) scales, in young fruitbodies densely arranged (sometimes covering the whole surface), in mature fruitbodies densely arranged at center, loosely arranged towards the margin, sometimes scales reaching the margin more pale, reddish brown (8D4), brownish violet (11D6), greyish red (11D5), greyish ruby (12E5) to greyish magenta (13E5, 14E5), margin in young specimens mostly inflexed, in mature specimens inflexed or straight, often undulate and/or orange colored; LAMELLAE adnate, crowded, yellow (3A6) to yellowish white (3A2); STIPE 40–90 × 7–12 mm central, cylindric, sometimes tapering upwards, straight to slightly curved, surface smooth to fibrillose, white, in mature specimens at some places/ or after pressing pastel yellow (3A4), with scarce greyish violet fibrils near the apex in a mature specimen, while in the same specimen with obscure pale violet felty covering when young; CONTEXT yellowish; BASIDIOMES growing in small groups.



Fig. 1. *Tricholomopsis flammula*: a–k. Basidiospores [a, b. ATHUM 9529; c, d. ATHUM 9543; e, f. ATHUM 9545; g–k. ATHUM 9528]; l. Cheilocystidia [ATHUM 9546]; m, n. Pleurocystidia [m. ATHUM 9543; n. ATHUM 9528]; o–q. Basidiomes [o, p. ATHUM 9546; q. ATHUM 9529]. Scale bars = 10 μ m.

BASIDIOSPORES (4.2–)4.9–8.1(–8.9) $\times$ (3–)3.1–4.2(–4.6) μm , $L_m = 6.5 \mu\text{m}$, $W_m = 3.6 \mu\text{m}$, $Q = 1.32\text{--}1.79\text{--}2.47$, ellipsoid to cylindrical, mostly oblong and obovoid, some phaseoliform, less often almost lacrymoid, smooth, hyaline, inamyloid, some with one guttule; BASIDIA 16–23 $\times$ 5–6 μm , clavate, narrowly clavate to cylindric, 4-spored; CHEILOCYSTIDIA (16–)25–60(–70) $\times$ (9–)11–21(–27) μm , forming a sterile edge, clavate, mostly broadly clavate; PLEUROCYSTIDIA (32–)38–60(–67) $\times$ (5–)6.5–9.5(–14.5) μm , abundant, irregularly cylindric, narrowly clavate, clavate, narrowly fusiform to fusiform, less often almost lageniform, utriform with tapering base, with yellow, golden-yellow, “oily” content; LAMELLAR TRAMA regular, hyphae 6–16 μm , some with yellow content; PILEIPELLIS a cutis of hyphae 5–10 μm wide, upper layer of hyphae with brownish-red intracellular granular pigment; CLAMP CONNECTIONS present.

HABITAT—natural, pure forests of *Abies cephalonica* Loudon, usually from 900–1500 m asl, on dead wood of *A. cephalonica*, mostly on fallen, decaying trunks.

SPECIMENS EXAMINED—GREECE: **ACHAIA, Mt. Klokos**, Natura 2000 protected area, on dead wood of *A. cephalonica*, 22.11.1999, leg. P. Tsopelas (ATHUM 9543); **ATTIKI, Mt. Parnitha National Park**, Mola, pure forest of *A. cephalonica*, on dead wood of *A. cephalonica*, 17.11.2004, leg. G. Athanasakis (ATHUM 9545); **FTHIOTIDA, Mt. Oiti National Park**, pure forest of *A. cephalonica*, on dead wood of *A. cephalonica*, 22.10.2013, leg. C. Chondralis (ATHUM 9528); **KEFALONIA ISLAND, Mt. Ainos National Park**, recreational park, 1470 m asl, pure forest of *A. cephalonica*, on fallen dead trunk of *A. cephalonica*, 12.10.2012, leg. M. Triantafyllou & Z. Gonou-Zagou (ATHUM 9546); recreational park, pure forest of *A. cephalonica*, 13.10.2013, leg. A. Tsasis & G. Tsasi (ATHUM 9547); Eza, 950 m asl, pure forest of *A. cephalonica*, on fallen dead trunk of *A. cephalonica*, 29.10.2012, leg. M. Triantafyllou (ATHUM 9529).

Tricholomopsis sulfureoides (Peck) Singer [as “*sulphureoides*”], Ann. Mycol.

41(1/3): 69 (1943)

FIG. 2

$\equiv$ *Agaricus sulfureoides* Peck, Ann. Rep. N.Y. State Mus. 23: 86 (1872)

= *Tricholomopsis osiliensis* Vauras, Folia Cryptog. Estonica 45: 87 (2009)

PILEUS 40–80 mm, convex to plano-convex, center depressed or with a broad umbo, surface smooth, greenish yellow (1A6), pastel yellow (1A4, 2A4), light yellow (2A5) to yellow (2A6), one fruitbody with greyish yellow (2B4) spots and grey brown tinges at umbo, pileus covered almost completely with fine, light yellow (3A5), greyish yellow (4C5) to golden (4C6) fibrils, margin mostly inflexed, often radially cracked, mature fruitbodies evolving orange, orange brown or grey brown spots (maybe after handling); LAMELLAE adnate sometimes with a decurrent tooth, medium crowded to crowded, pastel yellow

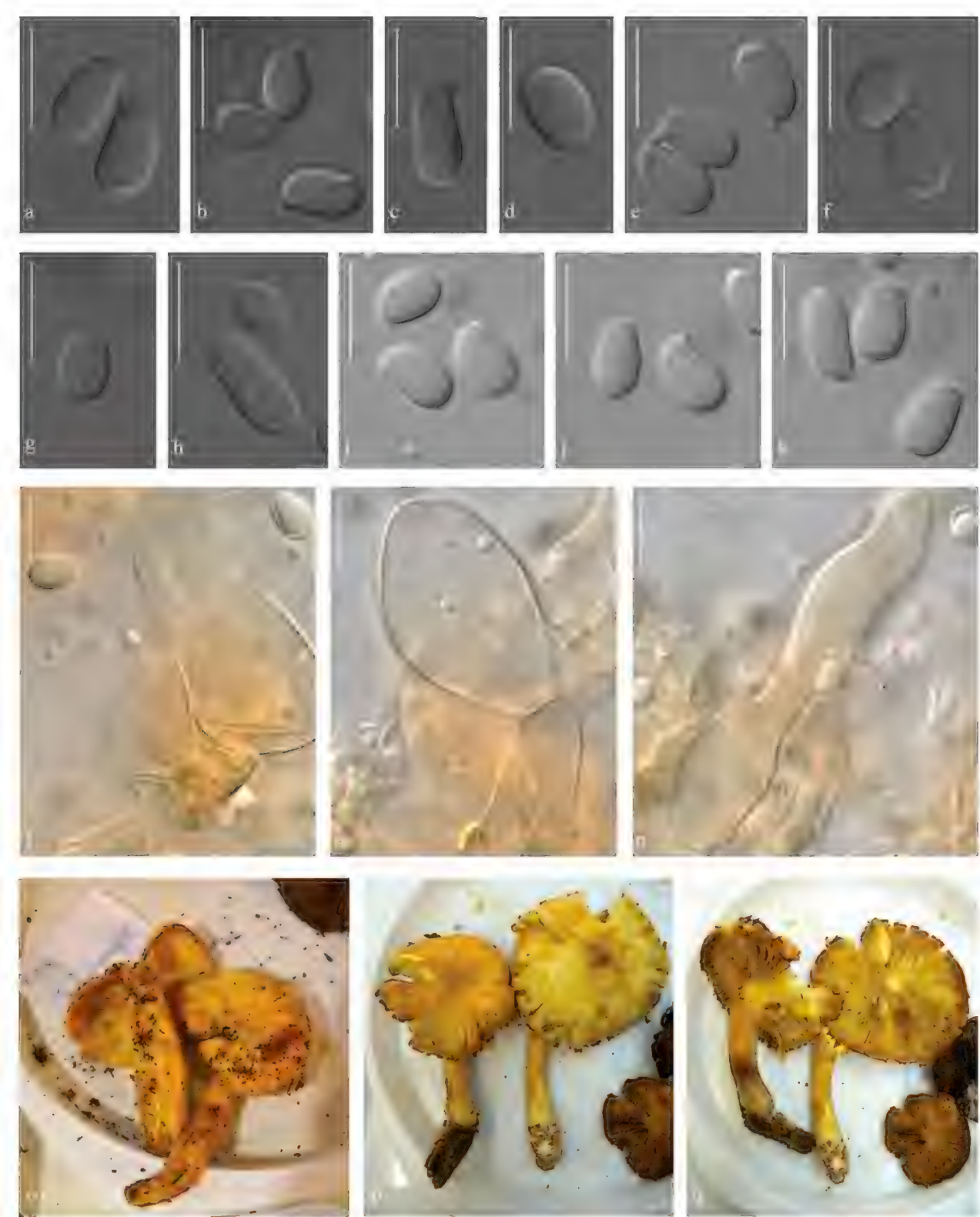


Fig. 2. *Tricholomopsis sulfureoides*: a–k. Basidiospores [a–e. ATHUM 9526; f–h. ATHUM 9548; i–k. ATHUM 9527]; l–n. Cheilocystidia [ATHUM 9527]; o–q. Basidiomes [o. ATHUM 9527; p, q. ATHUM 9548]. Scale bars = 10 μ m.

(1A4, 3A4) with greenish yellow (1A6) or greyish yellow (2B4) tints; STIPE 50–70 mm, cylindric, swollen at the median part or at base, slightly curved, almost concolorous with pileus, surface light yellow (1A5, 2A5), pastel yellow (1A4), yellow (2A6), greyish yellow (2B4), in one collection with scarce brown (5F4, 6F4) to dark brown (7F4–5) contrasting fibrils; CONTEXT yellowish; BASIDIOMES growing in small groups.

BASIDIOSPORES (5.7–)6.3–9.8(–11.4) $\times$ (3.7–)3.9–5.6(–6.1) μm , $L_m = 8 \mu\text{m}$, $W_m = 4.8 \mu\text{m}$, $Q = 1.34–1.68–2.34$, ellipsoid to cylindrical, mostly oblong and obovoid, a few phaseoliform, smooth, hyaline, inamyloid, some with one guttule; BASIDIA 32–40 $\times$ 5–6 μm , narrowly clavate to cylindric, 4-spored, sterigmata 3.5–5.5 μm long; CHEILOCYSTIDIA 22–75 $\times$ 12–26 μm , ovoid to obovoid, broadly fusiform to broadly clavate, mixed with a few cylindrical or irregularly lageniform, some with one (rarely two or more) projections, up to 15 μm , at/near apex; PLEUROCISTIDIA 40–65 $\times$ 5–6.5(–8) μm , frequent, irregularly cylindrical to cylindric-fusiform, often with constrictions, some with yellow content, few emerging deep from trama; LAMELLAR TRAMA regular; PILEIPELLIS a cutis of pale brown hyphae 3–7 μm broad; CLAMP CONNECTIONS present in all tissues.

HABITAT—natural, pure forests of *Abies cephalonica*, from 1400–1800 m asl, on dead wood of *A. cephalonica*.

SPECIMENS EXAMINED—**GREECE: ARCADIA, Mt. Mainalo**, Natura 2000 protected area, 1800 m asl, pure forest of *A. cephalonica*, on dead wood of *A. cephalonica*, 21.10.2001, leg. G. Prountzopoulos (ATHUM 9526); **FOKIDA-BOIOTIA, Mt. Parnassos National Park**, pure forest of *A. cephalonica*, on dead wood of *A. cephalonica*, 10.11.2012, leg. group of Foray & Z. Gonou-Zagou (ATHUM 9527); Varkos Despoti, 1400 m asl, pure forest of *A. cephalonica*, on stump of *A. cephalonica*, 7.11.2015, leg. I. Pyrri & A. Sergeantani (ATHUM 9548).

Phylogenetic analysis

Phylogenetic analysis of ten species of *Tricholomopsis* discloses four main clades, the two sister clades of *T. decora* and *T. badinensis* and two related but well-separated clades, the basal one comprising the species of southern hemisphere, while the other encompasses all remaining species. Greek specimens of *T. sulfureoides* cluster with other European and American *T. sulfureoides* specimens in a well-supported clade (FIG. 3). Concerning *T. flammula*, the phylogenetic tree reveals the same general topology as previous studies (Holec & Kolařík 2012; Olariaga & al. 2015). Therefore, the two sister subgroups that correspond to the “Western and Central European” and “Carpathian” clades of Holec & Kolařík (2012) are present as well (FIG. 3). The specimen from Pakistan is nested within the former clade. Interestingly, the

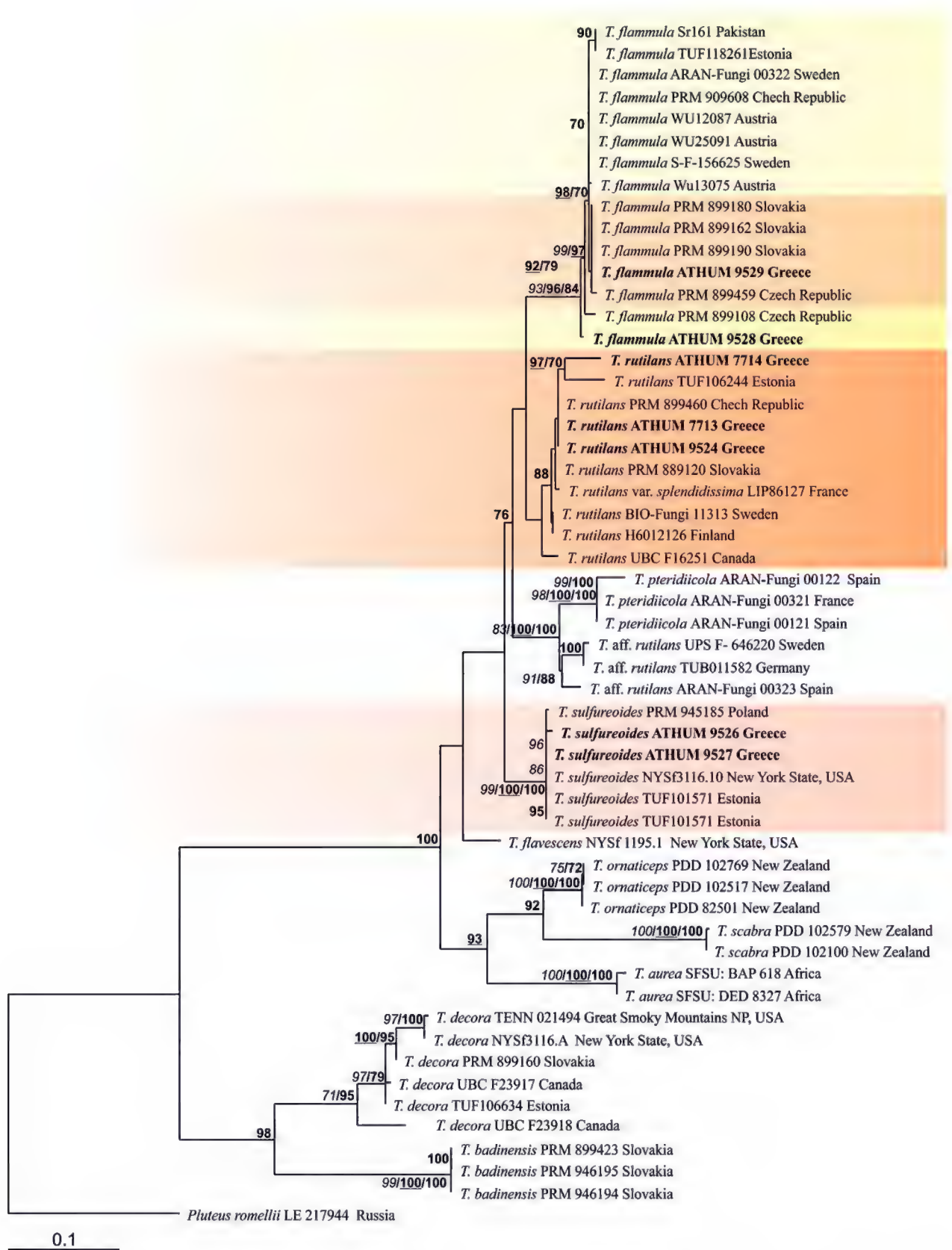


Fig. 3. Phylogenetic tree constructed from aligned DNA sequences of the ITS region as produced by ML. Sequences obtained during this study are presented in bold. Bootstrap values >70% for RAXML (numbers in bold) and NJ (numbers in italics), and posterior probabilities >0.95 for BI (numbers in bold underlined) are indicated at the nodes. *Pluteus romellii* LE 217944 was chosen as outgroup.

Greek specimen ATHUM 9529 is included in the “Carpathian” clade, whereas the Czech specimen PRM 899108 stands out of the two subclades forming a sister clade to them, and the Greek specimen ATHUM 9528 represents a basal clade to all.

Discussion

Tricholomopsis flammula

Tricholomopsis flammula was first described from France and is regarded as a rare species though it has a wide distribution in Europe and it is found also in Pakistan (Holec 2009; Holec & Kolařík 2011, 2012; Razaq & al. 2012). Most important characteristics of *T. flammula* that distinguish this species from the common *T. rutilans* are the yellowish stipe, typically without red or violet colors and the more ellipsoid spores, as it is also indicated by the Q values of spores for both species. The abundance of pleurocystidia in specimens of *T. flammula* was previously also used as a separating feature from *T. rutilans* (Krisai-Greilhuber & Voglmayr 2000; Holec 2009; Holec & Kolařík 2011). Olariaga & al. (2015) observed that pleurocystidia can be also found in specimens of the *T. rutilans* s.str. clade, indicating that this feature can no longer be highlighted when trying to separate *T. flammula* from *T. rutilans* s.str. Most Greek specimens of *T. rutilans* deposited at the Mycetotheca ATHUM possess no pleurocystidia, but in some specimens infrequent pleurocystidia are observed. Spores of Greek collections of *T. flammula* are rather narrow ($4.9\text{--}8.1 \times 3.1\text{--}4.2 \mu\text{m}$, $Q = 1.32\text{--}1.79\text{--}2.47$) and comparing them with the spores of Greek collections of *T. rutilans* ($((5.3\text{--})5.8\text{--}8.3(-9) \times (3.9\text{--})4.1\text{--}5.6(-6) \mu\text{m}$, $Q = 1.2\text{--}1.46\text{--}1.87$) it is confirmed that the more reliable distinguishing feature between the two species is the spore shape indicated by Q values.

Holec & Kolařík (2011, 2012) stated that there is molecular and morphological variation among specimens of *T. flammula*. In their study on the genus *Tricholomopsis* in Europe they noted that Slovak specimens of *T. flammula* possess more elongated spores than other European collections and develop scarce violet fibrils on stipe of mature basidiomes that can be more evident in young fruitbodies as a violet “felt”. In addition, molecular analysis showed that the Slovak specimens together with one Czech collection (PRM 899459) grouped together in the phylogenetic tree and this clade was characterized as “Carpathian” *T. flammula* subpopulation. Our molecular analysis supports the two sister subclades, the “Western and Central European” and the “Carpathian”, in the *flammula* clade. The specimen ATHUM 9529 is grouped within the “Carpathian” clade, whereas the ATHUM 9528 is a basal clade to

all specimens of the species. The Czech specimen PRM 899108 is excluded from the two subclades of the *flammula* clade forming a sister clade to it. The last two collections, when compared morphologically, reveal differences both in spore size and colors of the basidiomes, the Greek specimen having more elongated spores $(4.4\text{--})5.3\text{--}8.7(-8.9) \times (3\text{--})3.1\text{--}4.2(-4.6) \mu\text{m}$ vs $(5.2\text{--})5.5\text{--}6.4 \times 3.2\text{--}4.0 \mu\text{m}$) and densely arranged purple fibrillose scales mainly in the center of the pileus. They were not observed any 2-spored basidia that could justify the production of longer spores, as Holec & Kolařík (2011) mentioned for the Czech specimen PRM 899459. Spore size and shape vary slightly between the various Greek collections, as well as within the same collection. Nevertheless, all of them fit well in the “Carpathian” subclade because of the accordance of their mean $Q = 1.79$ with Holec & Kolařík’s (2012) data ($= 1.72\text{--}1.90$).

It is worth mentioning that sample ATHUM 9528, which seems to deviate phylogenetically, does not show any important morphological difference from other typical *T. flammula* specimens, except having slightly more elongated spores (TABLE 2). In addition, specimen ATHUM 9547 is the only one similar to the Slovak specimens in terms of the presence of scarce violet fibrils on the apex of the stipe of a mature fruitbody and the obscure pale violet felty covering of a young fruitbody (Holec & Kolařík 2012). Inferentially, there is morphological and genetic infraspecific variability that might reveal an allopatric speciation, but cannot justify the description of new taxa yet, not until new data are presented.

TABLE 2. Mean spore sizes of Greek collections of *Tricholomopsis flammula*

VOUCHER	VEGETATION	LOCALITY	LENGTH	WIDTH	L/W
ATHUM 9546	<i>A. cephalonica</i>	Mt. Ainos	6.1	3.6	1.72
ATHUM 9529	<i>A. cephalonica</i>	Mt. Ainos	6	3.5	1.69
ATHUM 9547	<i>A. cephalonica</i>	Mt. Ainos	6.2	3.5	1.78
ATHUM 9528	<i>A. cephalonica</i>	Mt. Oiti	7	3.6	1.93
ATHUM 9543	<i>A. cephalonica</i>	Mt. Klokos	6.3	3.7	1.69
ATHUM 9545	<i>A. cephalonica</i>	Mt. Parnitha	6.8	3.8	1.77

In Europe, *T. flammula* grows mostly on decaying trunks of conifers (*Abies*, *Picea*), occasionally also on dead wood of broadleaf trees and does not seem to have a preference over protected areas (Holec & Kolařík 2011, 2012). All

specimens studied were found on dead wood of *Abies cephalonica*, in *A. cephalonica* forests of Western, Central Greece, and the Peloponnese, in sites inside National Parks and Natura 2000 protected areas, the protection status of the latter being disputable. It is worth mentioning, that *T. flammula* is found in Mt. Ainos in the island of Kefalonia, which is the type locality of the endemic species *A. cephalonica*, the forests of which represent a Mediterranean mountainous habitat quite different from the above-mentioned Central and North European habitats. *Tricholomopsis flammula* is so far recorded only from *A. cephalonica* forests in Greece, while the closely related *T. rutilans* has been found growing also on dead wood of *Abies borisii-regis*. The number of samples indicates that the species seems to be rare, though widespread within the distribution area of pure *A. cephalonica* forests, where its appearance is rather uncommon. It is noteworthy, that pure *A. cephalonica* forests occur mainly in Central Greece and Peloponnese. Additionally, the species is reported from *Fagus* forests in Mt. Vasilitsa and Mt. Grammos, in Northwestern Greece (Konstantinidis 2006), where it is characterized as unusual. We did not have the chance to study specimens from the broadleaf forests, if there are any. To our knowledge, the Greek collections of *T. flammula* reveal the southernmost distribution of the species in Europe.

Tricholomopsis sulfureoides

Tricholomopsis sulfureoides was originally described from North America and in Europe was first recorded in Estonia quite recently as *T. osiliensis* (Vauras 2009). The species was recorded twice as *T. osiliensis* from Estonia on fallen trunk of *Picea abies* (L.) H. Karst. (Vauras 2009; Kalamees 2011) and from a virgin forest in Slovakia on *Abies alba* Mill. (Holec 2012b). Holec & al. (2019) referred to the presence of *T. sulfureoides* in Poland in five sites from the Białowieża virgin forest on fallen trunks of *P. abies*. They reported also two more collections of the species, one from Norway and one from Moscow in Russia. Dovana & Galbusera (2019) reported the occurrence of *T. sulfureoides* in Northern Italy. Interestingly, *T. sulfureoides* was recently proposed for assessment at the Global Fungal Red List Initiative and was preliminarily assessed as vulnerable (VU). In the same List the occurrence of the species in another four European countries is mentioned (Spain, France, UK, Switzerland) without any further details.

In 2012, during an educational foray for biology students, two yellowish fruitbodies belonging to *Tricholomopsis* were collected from a protected fir (*A. cephalonica*) forest in Central Greece (Mt. Parnassos National Park). Both

macromorphological and micromorphological characters fitted with the newly described species *T. osiliensis*, which is now considered to be conspecific with *T. sulfureoides* (Saar & Voitek 2015). After this finding, we examined a dried, previously unidentified *Tricholomopsis* specimen collected from Peloponnese (Mt. Mainalo; *A. cephalonica* forest) in 2001 and preserved in the Mycetotheca ATHUM, whose characteristics led to the same species. This specimen is currently the oldest collection of *T. sulfureoides* from Europe and the first record from Greece. Molecular identification confirmed our determinations. In 2015 a new sample of *T. sulfureoides* was collected in *A. cephalonica* forest of Mt. Parnassos National Park, at a different site from the one of the 2012 collections.

The study of microscopic characters in Greek specimens of *T. sulfureoides* reveals a high fluctuation in spore size and shape. The specimens possess a broader range of spore length in comparison to all other European collections, whereas the spore width is smaller reflecting thus higher Q values. *T. sulfureoides* is a species showing big variability in spore length measurements and consequentially in spore shape, both between different collections and within the same collection, as it was revealed from the Greek specimens, our observations being in accordance with those of Holec (2012b) and Holec & al. (2019). In addition, Holec & al. (2019) notified that the newly described species *T. badinensis* and *T. sulfureoides* have similar and overlapping microscopic characters though the spores of *T. badinensis* typically look slenderer than those of *T. sulfureoides*. All three Greek specimens of *T. sulfureoides* have spores with slightly higher Q values resembling more those of *T. badinensis*. This finding strengthens the conclusion of Holec & al. (2019) that there are no distinguishing microscopic characters between the two species to be used for accurate diagnosis. In TABLE 3 a comparison of spore sizes from European collections of *T. sulfureoides*, the American type collection of *T. sulfureoides*, and the Slovak species *T. badinensis* is given.

In Greek specimens two types of cheilocystidia are observed: from ovoid to obovoid, broadly fusiform to broadly clavate, but also cylindric or irregularly lageniform, some with usually one projection, up to 15 µm. Holec (2012b) described also clavate cystidia with such a projection, up to 30 µm from the Slovak collection, while Smith (1960) referred fingerlike projections in cheilocystidia of *T. bella* A.H. Sm., a species that he considered to form a species complex together with *T. thompsoniana* (Murrill) A.H. Sm. and *T. sulfureoides*. Pleurocystidia in studied material are frequent, some emerging deep from trama.

TABLE 3. Comparison of spore sizes of *Tricholomopsis sulfureoides* from Europe and USA (holotype) and *T. badinensis*.

VOUCHER	COUNTRY	SPORE MEASUREMENTS	REFERENCE
<i>Tricholomopsis sulfureoides</i>			
JV 26540F	Estonia	6.0–6.9–8.2 × 4.5–5.3–6.0 μm, Q = 1.2–1.33–1.5	Vauras 2009 [as <i>T. osiliensis</i>]
PRM 899184	Slovakia	8.0–9.6 × 5.6–6.0 μm, Q = 1.33–1.85 (mean = 1.52)	Holec 2012b [as <i>T. osiliensis</i>]
PRM 944836 PRM 945185 PRM 946096 PRM 946095 BRNU DD BIA17/401	Poland	6–8 × 4.5–6 μm	Holec & al. 2019
ATHUM 9526	Greece	6.9–9.6 × 4.1–5.6 μm, Q = 1.41–1.71–2.08	This study
ATHUM 9527	Greece	6.1–9.5 × 3.8–5.2 μm, Q = 1.44–1.72–2.34	This study
ATHUM 9548	Greece	6.1–10.1 × 4.2–5.9 μm, Q = 1.34–1.6–2.09	This study
NYSf 3116.10 [HT]	USA	5.5–8.5 × 3.5–6 μm, Q = 1.2–1.7 (mean = 1.4)	Saar & Voitek 2015
<i>Tricholomopsis badinensis</i>			
PRM 899423 PVKU 1007 PVKU 1439	Slovakia	7–9 × 4.5–6 μm, Q = 1.27–1.80 (mean = 1.46–1.60)	Holec & al. 2019

Fruitbodies of Greek collections of *T. sulfureoides* have yellow tones, almost concolorous pileus and stipe, without any trace of red or violet tinges. In mature basidiomata orange spots can be seen while scales on pileus are absent. Generally, the macromorphology of the specimens fits with the species descriptions, apart from collection ATHUM 9548, where the stipe is covered by scarce brownish contrasting fibrils, a feature which is not mentioned in descriptions of the European specimens. Moreover, it is important to mention that all fruitbodies evolved a dark purplish red coloration in pileus and stipe after drying.

T. sulfureoides grows on temperate and boreal locations of N. America and Europe on fallen trunks of *Picea* and *Abies*, in virgin, old growth forests (Slovak

and Polish collections) or at least in natural forests (Estonian collections; one of the two Estonian localities is a protected area) (Smith 1960; Vauras 2009; Holec 2012b; Holec & al. 2019). In Asia, there is only one specimen recorded, from China (Holec & al. 2019). Concerning Greek specimens, two of the three localities are situated inside a natural reserve in the oldest National Park of Greece in Mt. Parnassos, Central Greece. The third locality, where the earliest European collection of *T. sulfureoides* from Europe was found, is in Mt. Mainalo in Peloponnese, which, although it is included in the European Network Natura 2000 protected areas, it never had a stable status of protection. All Greek specimens were found on dead wood of *A. cephalonica*, the endemic fir of Greece, a fact which strengthens the assessment that *T. sulfureoides* grows in Europe on dead wood of conifers. Importantly, Greek collection sites seem to represent the most southern distribution of the species in Europe (there are no corresponding data from Spain). Our findings indicate that it is important to search for *T. sulfureoides* all over Europe and also worldwide, in order to understand its distribution and its ecological preferences, and thus give a satisfactory answer to the question posed by Vauras & al. (2012), why this species has not been recorded earlier in Europe.

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